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Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum

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Abstract

The 5-HT receptor mediating postjunctional relaxation of precontracted guinea-pig ileum has been characterized using several agonists and antagonists. Substance P precontracted tissues were potently relaxed by 5-HT (5-hydroxytryptamine, serotonin), 5-CT (5-carboxamidotryptamine) and several other indoles. The rank order of potency, with pEC_{50} values in parentheses, was 5-CT (7.6) > 5-methoxytryptamine (5.7) > 5-HT (5.5) > α-methyl-5-HT (4.7) > 2-methyl-5-HT (< 4.0) = tryptamine (< 4.0) = *N,N*-dimethyl-tryptamine (< 4.0) = *N,N*-dimethyl-5-HT (< 4.0) = dipropyl-5-CT (< 4.0) = sumatriptan (< 4.0). 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin) acted as a potent (6.3), but partial, agonist with respect to 5-HT. The responses to 5-CT were antagonized by several compounds with the following rank order of affinity, with pK_B values in parentheses: LSD (lysergic acid diethylamide; 8.1) = mesulergine (7.8) > methysergide (7.6) = spiperone (7.6) > clozapine (7.3) ≫ (−)-pindolol (< 6.0) > ketanserin (< 6.0) = ondansetron (< 6.0) = GR 113808 ([1-(2-methane-sulphonamido-ethyl)-piperidin-4-yl]-methyl-indole-3-carboxylate maleate; < 6.0). The relaxant responses to 5-HT were also resistant to tetrodotoxin. These data are consistent with a functional 5-HT receptor, mediating relaxation of guinea-pig ileum, which exhibits an operational profile similar to that of the cloned guinea-pig 5-ht_7 receptor. This study, therefore, provides evidence for a functional correlate of the 5-ht_7 gene product.

Keywords: Ileum, guinea-pig; 5-ht_7 receptor; Smooth muscle relaxation

1. Introduction

A putative 5-HT receptor sequence, representing a possible mammalian homologue of the drosophila 5-HT ($5\text{-}HT_{dro1}$) receptor (Hen, 1993), has been cloned from mouse, rat, guinea-pig and human cDNA libraries (Plassat et al., 1993; Lovenberg et al., 1993; Ruat et al., 1993; Shen et al., 1993; Meyerhof et al., 1993; Tsou et al., 1994; Bard et al., 1993; see Boess and Martin, 1994, for review). The receptor is presently designated as 5-ht_7 (Hoyer et al., 1994): the lowercase appellation emphasizing the lack of unambiguous endogenous correlates (Kenakin et al., 1992). Transfection of receptor cDNAs into cells results in expression of a 5-HT receptor that couples positively to adenylyl cyclase (see Hoyer et al., 1994, for review). The transfected 5-ht_7 receptor is potently stimulated by 5-CT (5-carboxamidotryptamine), less so by 5-HT (5-hydroxytryptamine, serotonin) and competitively antagonized by LSD (lysergic acid diethylamide), methiothepin, metergoline and mesulergine (see Boess and Martin, 1994; Eglen et al., 1994 for reviews). The receptor is also antagonized by clozapine, a characteristic that, operationally, serves to distinguish the receptor from the $5\text{-}HT_1$ family. Structurally, the 5-ht_7 receptor lacks an asparagine at residue 371. This feature, with the ability to activate rather than inhibit adenylyl cyclase activity, serves to distinguish the clone from $5\text{-}HT_{1A}$ receptors. It is interesting, therefore, which 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin), generally considered a selective $5\text{-}HT_{1A}$ agonist (Middlemiss and Fozard, 1983), acts as a partial agonist at the 5-ht_7 receptor clone (Lovenberg et al., 1993; Tsou et al., 1994).

Several 'atypical' 5-HT receptors, functionally characterized in smooth muscle, may represent endogenous

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correlates of this receptor clone (Bard et al., 1993; Tsou et al., 1994; Martin and Wilson, 1995). One candidate is a 5-HT receptor in guinea-pig isolated ileum, stimulation of which mediates muscle relaxation (Feniuk et al., 1983; Kalkman et al., 1986). The receptor is highly sensitive to activation by 5-CT and competitively antagonized by methysergide ($pA_2 = 7.5$), metergoline ($pK_B = 7.9$), mesulergine ($pK_B = 7.9$) and spiperone ($pK_B = 7.7$; Feniuk et al., 1983; Kalkman et al., 1986). RU 24969 fails to elicit a response, suggesting that 5-HT_{1A} or 5-HT_{1B} receptors are uninvolved (Kalkman et al., 1986). The receptor is probably postjunctionally located, since tetrodotoxin does not affect the relaxant responses to 5-HT (Feniuk et al., 1983). In this respect, the site differs from 5-HT_{1A}, 5-HT_3 and 5-HT_4 receptors, all of which are prejunctionally located in guinea-pig ileum. Radioligand binding studies, using [^{125}I]LSD in guinea-pig ileal membranes, have revealed a specific binding site with high affinity toward metergoline ($pK_i = 7.5$), mesulergine ($pK_i = 7.4$), methysergide ($pK_i = 8.5$) and iodo-LSD ($pK_i = 8.8$; Kalkman et al., 1986).

Taken together, these data indicated a functional receptor, positively correlated with a binding site, which exhibits some pharmacological similarities to the 5-ht_7 receptor clone. Concordantly, Northern blot analysis has shown that mRNA for the 5-ht_7 receptor is expressed in both guinea-pig and human ileal smooth muscle (Tsou et al., 1994; Bard et al., 1993), while Reddy et al. (1994) have shown that 5-HT enhances adenylyl cyclase activity in guinea-pig ileal slices.

The aim of the present study, therefore, was to further characterize the 5-HT receptor in guinea-pig ileum mediating relaxation, using several agonists and antagonists. The relaxant effects of several 5-HT receptor agonists were evaluated by their ability to attenuate a contracture induced by either histamine or substance P. In the first series of studies, histamine was used to repeat earlier work by Feniuk et al. (1983) and Kalkman et al. (1986). However, preliminary studies showed that several 5-ht_7 receptor ligands, including some ergot derivatives and indole analogues, possess affinity for the histamine H_1 receptor. Consequently, substance P was used as a spasmogen in the studies using compounds to antagonize the putative 5-HT receptor.

2. Materials and methods

2.1. Preparation and experimental design

Male, Dunkin-Hartley, guinea-pigs (250–350 g) were killed by CO_2 asphyxiation and the proximal ileum removed. The ilea were flushed with warm (37° C) Krebs solution of the following composition (mM); NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.2 and glucose 11.2. Whole ileal segments, approx. 2 cm in length, were suspended under 1.0 g tension in gassed (95% O_2/5% CO_2) Krebs solution and maintained at pH 7.4, 37° C. The Krebs solution also contained cocaine (10 μM) and corticosterone (10 μM), to prevent neuronal and extraneuronal uptake, respectively, as well as pargyline (5 μM) to inhibit monoamine oxidase activity. Indomethacin (1 μM) was included to inhibit cyclooxygenase activity while atropine (1 μM) was added to antagonize cholinergically mediated contractions, arising from activation of 5-HT_3 and 5-HT_4 receptors (see Craig et al., 1990 for references).

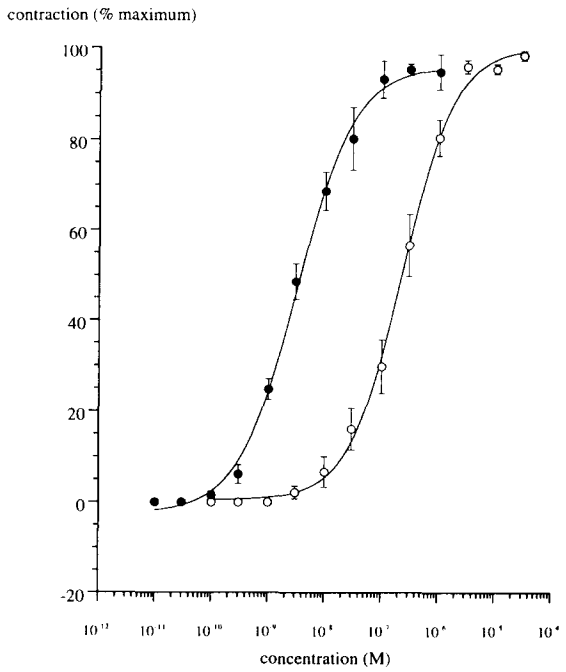

Fig. 1. Concentration-response curves to substance P (closed circles) and histamine (open circles) in guinea-pig isolated ileum. Values shown are mean, with vertical deflections representing S.E.M., $n = 10$.

In preliminary experiments, tissues were exposed to a single concentration of histamine (1 μM), to obtain a sustained contracture upon which relaxant responses to cumulative additions of 5-HT receptor agonists could be seen (Feniuk et al., 1983). However, this proved unreliable since, in many tissues, the tone of the preparation gradually declined over the succeeding 20 min and relaxant responses to 5-HT were difficult to distinguish from 'fade' of the contractile tone. An alternative protocol (Fig. 1) was, therefore, used, which was modified from that described by Bond and Clarke (1988). Thus, after an equilibration period of 60 min, tissues were contracted with either histamine (1 μM) or substance P (30 nM) for 30 s, on a 5 min dose-cycle.

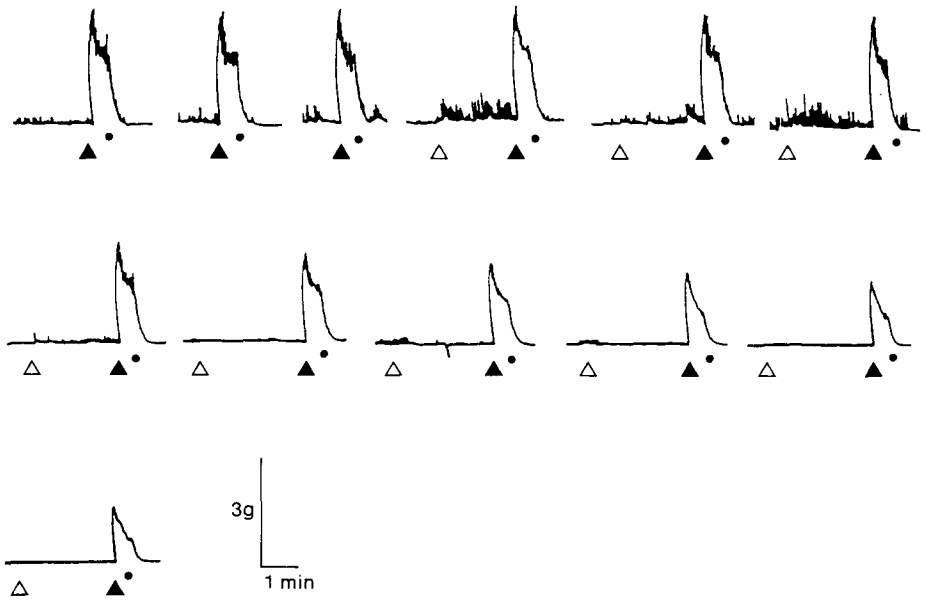

Fig. 2. Representative polygraph trace showing the functional antagonism (relaxation) of 5-CT on contractures elicited by substance P in guinea-pig isolated ileum.

Preliminary experiments had shown that these concentrations elicited equivalent increases in isometric tension (3.2–3.5 g) and approximated to the EC_{80} value for each agonist. The tissues were 'pulsed' in this manner throughout the experiment. Once stable contractures were attained, 5-HT receptor agonists were added 3 min before the addition of spasmogen and concentration-response curves established, on a noncumulative basis, using incremental concentrations spaced at 0.5 log units. Preliminary experiments had shown that 3 min was sufficient to allow development of a maximal inhibition of the contracture by 5-HT. In each experiment, a paired tissue control was run in parallel to correct for potential changes in sensitivity to the spasmogen. In practice, however, reproducible contractures to either spasmogen were generally attained throughout the duration of the experiment.

Selective agonists and antagonists to define the putative 5-ht_7 receptor are unavailable (Eglen et al., 1994). Desensitization was, therefore, used to discriminate between compounds acting as 5-HT receptor agonists, histamine H_1 receptor antagonists and/or nonspecific smooth muscle relaxants. The agonist studies were consequently repeated in the presence of 3 μM 5-CT, following equilibration with 5-CT for 60 min, during which time the bathing solution was changed at 15 min intervals. This concentration of 5-CT had no effect on the histamine contracture per se but abolished relaxant responses emanating from 5-HT receptor stimulation. Relaxations persisting under these conditions were, therefore, presumed to be due to reasons other than 5-HT receptor stimulation. Kalkman et al. (1986) have indicated that 8-OH-DPAT (a partial agonist at 5-ht_7 receptors, Lovenberg et al., 1993), almost maximally (80–100%), relaxed the histamine precontracted ileum. This is in contrast to maximal responses

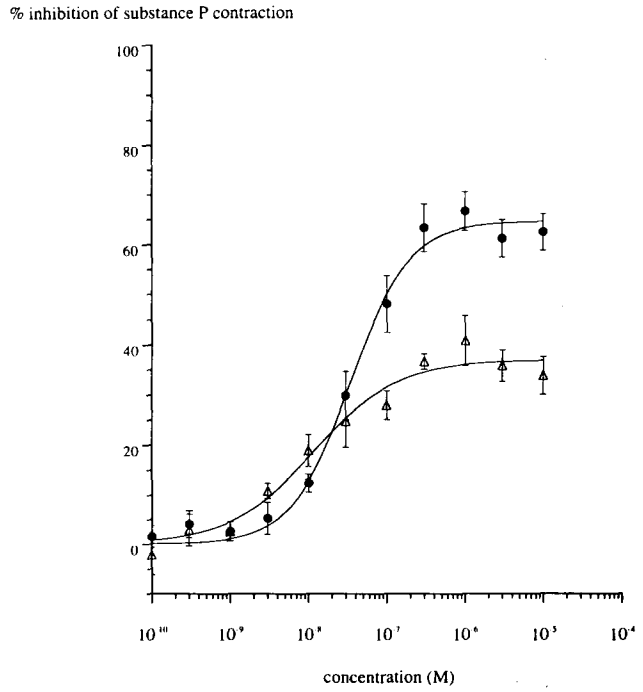

Fig. 3. Concentration-relaxation curves to isoprenaline (closed circles) and 5-carboxamidotryptamine (5-CT; open triangles) in substance P contracted guinea-pig ileum. Values are mean, with vertical deflections representing S.E.M., $n = 6–10$. Substance P (30 nM) was added at times indicated by the closed triangles, 5-CT at times indicated by the open triangles and washes at times indicated by the closed circles.

Table 1
Agonist potencies (pEC_{50}) at 5-HT receptors mediating relaxation of histamine-contracted guinea-pig, isolated ileum

	Control		Desensitized [a]	
	pEC_{50}	% Relaxation	pEC_{50}	% Relaxation
5-CT	6.9 ± 0.22	47 ± 7.6	NR	–
5-HT	5.0 ± 0.22	47 ± 7.0	NR	–
α-Methyl-5-HT	4.5 ± 0.3	34 ± 6.0	NR	–
5-Methoxytryptamine	< 4.5	–	NR	–
2-Methyl-5-HT	NR	–	NR	–
Tryptamine	NR	–	NR	–
Sumatriptan	NR	–	NR	–
N,N-Dimethyl-tryptamine	6.0 ± 0.4	106 ± 12	5.9 ± 0.04	93 ± 2.6
Dipropyl-5-CT	5.2 ± 0.05	94 ± 4.2	5.1 ± 0.08	86 ± 6.4
N,N-Dimethyl-5-HT	4.7 ± 0.08	80 ± 7.7	4.6 ± 0.09	81 ± 9.2
Isoprenaline	7.34 ± 0.06	71.2 ± 2.8	ND	ND

Values are mean ± S.E.M., $n = 6–10$. NR – No response. [a] Desensitization studies were conducted in the presence of 3 μM 5-CT, following an equilibration period of 60 min. ND – not determined.

to either 5-HT or 5-CT (approx. 50% relaxation). Consequently, the relaxant effects of this compound were assessed against substance P contractures only.

2.2. Antagonist studies

Substance P was used as the spasmogen in all antagonist studies, with the exception of studies using methysergide, which has been shown to competitively antagonize relaxant responses to 5-CT (Feniuk et al., 1983; Kalkman et al., 1986). In the present study, the tissues were equilibrated with antagonists 60 min before construction of a concentration-response curve to 5-CT. During the final 15 min of this equilibration period, the tissues were pulsed with substance P in order to attain a stable series of contractures, as described above. To eliminate the possibility of 5-CT-induced desensitization, only one concentration-response curve to 5-CT was established in each tissue. The dextral shift in the 5-CT concentration-response curve in the presence of the antagonist was compared to a paired tissue run in parallel, without antagonist. In this manner, a concentration ratio was established and used to derive apparent antagonist affinities.

2.3. Analysis of results

Tissue responses were recorded as gram changes in isometric tension. The relaxant responses were expressed as a percentage of the initial contractures to the spasmogen or a percentage reduction in the height of the contracture. The values shown are the mean ± S.E.M., where n refers to the number of animals. Where appropriate, agonist potencies (pEC_{50}) were estimated by the relationship of Parker and Waud (1971), by nonlinear iterative curve fitting procedures (Leung et al., 1992). In several cases, the responses did not attain a clear maximum and no attempt was made to calculate the potency. Antagonist affinities (pK_B) were estimated by the method of Furchgott (1972),

$$pK_B = -\log(\text{antagonist concentration M}) / \text{concentration ratio} - 1.$$

Where appropriate, pA_2 values were estimated using the method of Arunlakshana and Schild (1959), from three antagonist concentrations. Statistical differ-

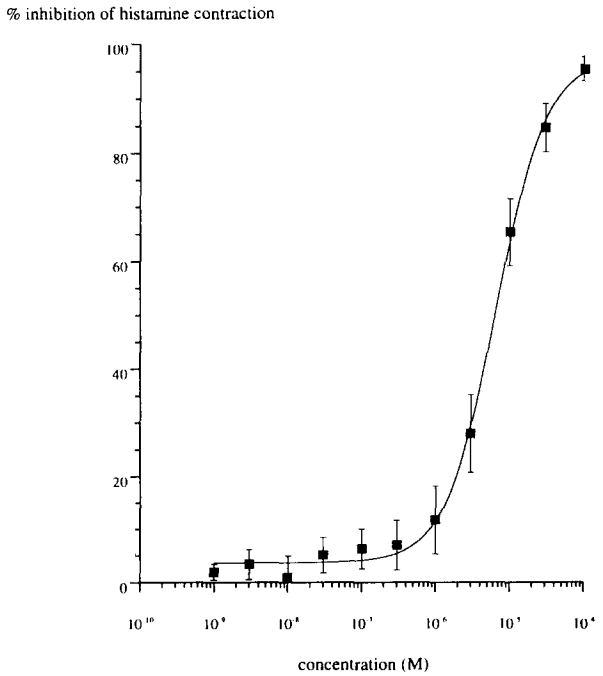

Fig. 4. Concentration-relaxation curves to dipropyl-5-CT (dipropyl-5-carboxamidotryptamine; closed squares) in substance P contracted guinea-pig ileum. Values are mean, with vertical deflections representing S.E.M., $n = 6–10$.

Table 2
Agonist potencies (pEC_{50}) at 5-HT receptors mediating relaxation of substance P-contracted guinea-pig, isolated ileum

	pEC_{50}	% Relaxation
5-CT	7.6±0.10	44±3.2
8-OH-DPAT	6.3±0.21	26±5.2
5-Methoxytryptamine	5.7±0.12	43±5.9
5-HT	5.5±0.26	34±2.7
α-Methyl-5-HT	4.7±0.17	44±7.0
2-Methyl-5-HT	NR	–
Tryptamine	NR	–
Sumatriptan	NR	–
N,N-Dimethyl-tryptamine	NR	–
Dipropyl-5-CT	NR	–
N,N-Dimethyl-5-HT	NR	–
Isoprenaline	7.51±0.08	73.4±3.6

Values are mean±S.E.M., $n = 6–10$. NR – no response.

ences were assessed using Student's *t*-test, with $P < 0.05$ being considered significant.

2.4. Drugs

All compounds, with the exception of those listed below were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Research Biochemicals (Natick, MD, USA). Methysergide was generously donated by Sandoz (Basel, Switzerland). 5-Carboxamidotryptamine maleate, GR 113808 ([1-(2-methanesulphonamido-ethyl)-piperidin-4-yl]-methyl-indole-3-carboxylate maleate) and ondansetron were synthesized in the Institute of Organic Chemistry, Syntex Discovery Research (Palo Alto, CA, USA). All compounds were dissolve in distilled water at a concentration of 3 mmol/l.

3. Results

3.1. Agonists

Histamine and substance P induced concentration-dependent contractions of guinea-pig isolated ileum (Fig. 1). The responses to either substance P and histamine appeared to be reproducible, in that construction of two consecutive curves to both of these agonists, with 30 min allowed between curves, showed no significant change in potency or maximal response (data not shown). Concentration-dependent relaxations, i.e. inhibition of the histamine or substance P contractures, were seen with 5-HT, 5-CT or isoprenaline (an agonist that elicits relaxation through β_3-adrenoceptors (Bond and Clarke, 1988, Reddy et al., 1994; Figs. 2 and 3; Table 1). The magnitude of relaxant responses to isoprenaline was greater than those seen with either 5-HT or 5-CT (Fig. 3). *N,N*-Dimethyltryptamine, *N,N*-dimethyl-5-HT and dipropyl-5-CT (Fig. 4) induced concentration-dependent relaxations, with maximal responses much greater (100%) than that seen with either isoprenaline, 5-CT or 5-HT (40–50%). No relaxant responses were seen to 2-methyl-5-HT, tryptamine or sumatriptan (10 nM–30 μM). After equilibration (60 min), 5-CT abolished subsequent relaxant responses to 5-CT, 5-HT, α-methyl-5-HT and 5-methoxytryptamine. In contrast, relaxant responses to dipropyl-5-CT, *N,N*-dimethyl-tryptamine or *N,N*-dimethyl-5-HT were unaffected (Table 1).

Similar relaxant responses to 5-CT, 5-HT and isoprenaline were seen when substance P, rather than histamine, was used to precontract the tissue (Table 2). Again, no relaxant response was seen with 2-methyl-5-HT, tryptamine or sumatriptan. In these studies, 8-OH-DPAT acted as a partial agonist, eliciting 26% relaxation of the substance P contracture (Table 2). In contrast to the studies described above using tissues precontracted with histamine, *N,N*-dimethyl-tryptamine, *N,N*-dimethyl-5-HT or dipropyl-5-CT did not cause a relaxation.

3.2. Antagonists

Relaxant responses to 5-CT were surmountably antagonized by methysergide, mesulergine, LSD, spiperone and clozapine (Table 3). Analysis of antagonism of these responses to 5-CT by methysergide revealed a pA_2 value of 7.6 ± 0.08 and a Schild slope, which was not significantly different to unity, of 0.91 ± 0.05. (−)-Pindolol, ketanserin, ondansetron and GR 113808, at 1 μM, were without effect on the concentration-response curve to 5-CT (Table 3). Tetrodotoxin (1 μM) was also without effect on relaxant responses to 5-HT or 5-CT.

Table 3
Antagonist affinities (pK_B) at 5-HT receptors mediating relaxation of guinea-pig, isolated ileum

Antagonist	pK_B	Feniuk et al. (1983) pA_2	Kalkman et al. (1986) pA_2
LSD	8.1±0.4	–	8.1 [a]
Mesulergine	7.8±0.5	–	7.9
Methysergide	7.6±0.2	7.4	6.9
Spiperone	7.6±0.3		7.7
Clozapine	7.3±0.3		
(−)-Pindolol	< 6.0		
Ketanserin	< 6.0		
Ondansetron	< 6.0		
GR 113808	< 6.0		

Values are mean±S.E.M., $n = 6–8$. The control potency (pEC_{50}) for 5-CT was 7.76±0.16, which induced 44±3.2% relaxation of the substance P contracture. [a] pD'_2 value for iodo-LSD, which acted in a noncompetitive fashion (Kalkman et al., 1986).

None of the compounds studied affected the contractile response to substance P (data not shown).

4. Discussion

A 5-HT receptor mediating relaxation of guinea-pig ileum was described by Feniuk et al. (1983) and Kalkman et al. (1986) to be highly sensitive to activation by 5-CT and competitively antagonized by methysergide. Nonetheless, in the limited number of studies performed at this site (see Introduction for references), 5-CT was more potent than 5-HT, suggesting that the receptor was either a 5-HT_1 or 5-ht_7 receptor (Hoyer et al., 1994). In the present study we have confirmed and extended these data, using two different protocols. The methods described herein employed isometric, rather than isotonic, recording of changes in tension. This difference may explain why no sustained tone was seen in the present study to a contracture by histamine, in contrast to the studies of Feniuk et al. (1983) who used isotonic recording.

In the present study, both of these agonists relaxed the tissue in a concentration-dependent manner, and the maximal response to each agonist was equivalent, but less than that to isoprenaline. The potency of isoprenaline (pEC_{50} = 7.3–7.6) was close to that observed by Bond and Clarke (1988) (pEC_{50} = 8.0) or Reddy et al. (1994) (pEC_{50} = 7.3) using similar protocols in guinea-pig ileum. The lower maximal response to 5-CT or 5-HT, in comparison to isoprenaline, suggested that relaxations to these agonists did not occur by a 'tyramine-like' action of these indoles, acting to release noradrenaline; a suggestion also supported by the lack of effect of (−)-pindolol. It is of interest that the maximal response to 5-HT was less than that of 5-methoxytryptamine or 5-CT. The reason for this difference is unclear, although the methodology used may have affected determination of maximal responses. Indeed, previous studies (Feniuk et al., 1983; Kalkman et al., 1986), using 5-HT or 5-CT to relax a sustained contracture evoked by histamine, show that both compounds acted as full agonists. It is possible that at this ileal receptor, 5-HT acted as a partial agonist, at least with respect to other indoles. However, this is considered to be an unlikely explanation, given the recent finding that, at putative 5-ht_7 receptors in rabbit femoral vein, 5-HT and 5-CT acted as full agonists (Martin and Wilson, 1995).

The potency of 5-HT obtained using histamine-contracted tissues (pEC_{50} = 5.5) was less than that reported previously (pEC_{50} values = 5.9 and 6.7; Feniuk et al., 1983 and Kalkman et al., 1986, respectively). The reasons for this discrepancy are unclear, although the age of the animals (Kalkman et al., 1986) or the spasmogen used to elevate initial tone, may be factors. For example, the potency of 5-CT in substance P-contracted tissues was higher than in histamine-contracted tissues, even though the degree of developed tension was similar (3.0–3.2 g). This finding, together with the low affinity of most 5-HT ligands for the tachykinin NK_1 receptor in guinea-pig ileum, provides a good reason to use substance P, rather than histamine, as a spasmogen in the bioassay.

The high potency of 5-CT, and low potency of α-methyl-5-HT (this study), support data by Kalkman et al. (1986) and Feniuk et al. (1983), respectively, suggesting that 5-HT_2 receptors were uninvolved. Sumatriptan, tryptamine and 2-methyl-5-HT were inactive which is consistent with a lack of involvement of 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F} or 5-HT_3 receptors (see Hoyer et al., 1994, for review). Kalkman et al. (1986) have reported a lack of response to RU 24969, indicating an absence of 5-HT_{1A} receptors and similar conclusions may be drawn, from the present study, using dipropyl 5-CT, also a potent 5-HT_{1A} agonist (see Eglen et al., 1992, for references). This compound elicited complete relaxation of the tissue, greater than seen with the endogenous agonist, 5-HT. However, this finding, and the fact that these responses of dipropyl-5-CT were refractory to desensitization by 5-CT (Table 1), suggest a response mediated by other mechanisms. Similar conclusions may be drawn regarding the effects of *N,N*-dimethyl-tryptamine or *N,N*-dimethyl-5-hydroxytryptamine. Therefore, the rank order of potency of indole agonists *acting at the 5-HT receptor* was: 5-CT > 5-methoxytryptamine = 5-HT > α-methyl-5-HT > 2-methyl-5-HT = tryptamine = *N,N*-dimethyl-tryptamine = *N,N*-dimethyl-5-HT = dipropyl-5-CT = sumatriptan = inactive. This agonist rank order, therefore, suggests a site distinct from 5-HT_1, 5-HT_2, 5-HT_3 or 5-HT_4 receptors (Eglen et al., 1992). Further evidence to support this conclusion was obtained studies with antagonists; generally considered more appropriate tools for receptor classification (Kenakin et al., 1992).

Relaxant responses to 5-CT were antagonized by several antagonists, with the following rank order of affinities (Table 3) obtained: LSD = mesulergine > methysergide > spiperone > clozapine ≫ (−)-pindolol > ketanserin = ondansetron = GR 113808 = < 6.0. These pK_B values were in good agreement with those values reported in the literature (Table 3) and also to those values estimated using Schild regression analysis (this study; Feniuk et al., 1983; Martin and Wilson, 1995). This may suggest that, although the apparent affinity values were obtained using a single concentration of antagonist, they represent good estimates of a competitive interaction. It should be noted, however, that at putative 5-ht_7 receptors mediating relaxation of rabbit femoral vein, methysergide (pA_2 = 7.8) acted as a unsurmountable antagonist, while spiperone (pK_B = 7.8) acted as a surmountable antagonist (Martin and

Wilson, 1995). Nonetheless, the apparent affinities were similar to those estimated in the present study (Table 3).

Several conclusions may be drawn concerning the pharmacological nature of this site. Thus the relatively low affinity of this receptor for (−)-pindolol (this study) or propranolol (Kalkman et al., 1986) suggests a dissimilarity from 5-HT_{1A} or 5-HT_{1B} receptors, in agreement with the lack of agonism seen with dipropyl-5-CT (this study; see above) or RU 24969 (Kalkman et al., 1986). The low affinity of ketanserin (this study) and cyproheptadine (Feniuk et al., 1983) concurs with the low agonist potency of α-methyl-5-HT, indicating that the receptor was dissimilar from 5-HT_2 receptors. The lack of agonism seen with 2-methyl-5-HT supports the lack of antagonism with ondansetron (this study) and indicates an absence of 5-HT_3 receptor stimulation. The lack of antagonism by GR 113808 indicates that 5-HT_4 receptors were uninvolved.

Taken together, the site was pharmacologically positively defined by a moderate affinity toward LSD, mesulergine, methysergide and spiperone; 5-CT acting as a potent agonist and 8-OH-DPAT as a partial agonist. This operational profile is similar to the binding profile developed at guinea-pig 5-ht_7 receptors (Tsou et al., 1994) including the apparent affinity for clozapine (Table 3). There were some important discrepancies, however. Cloned 5-ht_7 receptors, from all species so far studied, bind 5-CT with high affinity (see Introduction for references). Although this indole was the most potent agonist observed, there was a difference between the *affinity* of this and other indoles ($pK_i = 8.0–9.5$) at cloned 5-ht_7 receptors and the *potency* observed at the site through which the ileum is relaxed ($pEC_{50} = 5.7–7.6$; this study; Feniuk et al., 1983; Kalkman et al., 1986). A low affinity of 5-HT and 5-CT ($pK_A = 5.7$ and 6.7, respectively) has also been reported at putative 5-ht_7 receptors mediating relaxation of rabbit femoral vein (Martin and Wilson, 1995). At present, there is no definitive explanation for these discrepancies, but they highlight the need for a functional bioassay of the 5-ht_7 receptor in order to validate data from radioligand binding studies.

In conclusion, these data are consistent with activation of a postjunctional 5-HT receptor that, operationally, has some similarities to 5-ht_7 receptors. The guinea-pig ileum, under the conditions described in the present study, thus provides a simple preparation for this putative 5-HT receptor, particularly when substance P is used to elevate tone. As pointed out by Feniuk et al. (1983), definitive characterization of the receptor mediating the response awaits the development of a selective antagonist. In the absence of such a compound, selective desensitization with 5-CT may provide a means to discriminate the action of putative 5-ht_7 agonists.

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